Targeting Heme-Oxidized Soluble Guanylate Cyclase in Experimental Heart Failure

Guido Boerrigter, Lisa C. Costello-Boerrigter, Alessandro Cataliotti, Harald Lapp, Johannes-Peter Stasch, John C. Burnett, Jr

Abstract—Soluble guanylate cyclase is a heterodimeric enzyme with a prosthetic heme group that, on binding of its main ligand, NO, generates the second messenger cGMP. Unlike conventional nitrovasodilators, the novel direct NO- and heme-independent soluble guanylate cyclase activator BAY 58-2667 is devoid of non-cGMP actions, lacks tolerance development, and preferentially activates NO-insensitive heme-free or oxidized soluble guanylate cyclase. BAY 58-2667, therefore, represents a novel therapeutic advance in mediating vasodilation. To date, its cardiorenal actions in congestive heart failure (CHF) are undefined. We, therefore, hypothesized that BAY 58-2667 would have beneficial preload- and afterload-reducing actions in experimental severe CHF together with renal vasodilating properties. We assessed the cardiorenal actions of intravenous administration of 2 doses of BAY 58-2667 (0.1 and 0.3 μg/kg per minute, respectively) in a model of tachypacing-induced severe CHF. In CHF, BAY 58-2667 dose-dependently reduced mean arterial, right atrial, pulmonary artery, and pulmonary capillary wedge pressure (from baseline 19±1 to 12±2 mm Hg). Cardiac output (2.4±0.3 to 3.2±0.4 L/min) and renal blood flow increased. Glomerular filtration rate and sodium and water excretion were maintained. Consistent with cardiac unloading, atrial and B-type natriuretic peptide decreased. Plasma renin activity (P=0.31) and aldosterone remained unchanged (P=0.19). In summary, BAY 58-2667 in experimental CHF potently unloaded the heart, increased cardiac output and renal blood flow, and preserved glomerular filtration rate and sodium and water excretion without further neurohumoral activation. These beneficial properties make direct soluble guanylate cyclase stimulation with BAY 58-2667 a promising new therapeutic strategy for cardiovascular diseases, such as heart failure. (Hypertension. 2007;49:1128-1133.)

Key Words: soluble guanylate cyclase ■ heart failure ■ drugs ■ oxidant stress ■ BAY 58-2667

Nitric oxide–sensitive soluble guanylate cyclase (sGC) plays an important role in cardiovascular regulation. It consists of an α and a β subunit, the latter of which has a binding site for a prosthetic heme group. Binding of NO to the ferrous heme iron (Fe2+) activates the enzyme and leads to conversion of guanosine triphosphate to the second messenger cGMP, which promotes various actions, such as vasodilation, inhibition of platelet aggregation, and growth inhibition.1 Importantly, cardiovascular disease is frequently associated with impaired NO–sGC–cGMP signaling, a condition that has also been termed “endothelial dysfunction” and that is associated with worse outcomes.2,3

Most recently, Stasch et al4 have provided seminal new insights into the molecular mechanisms of endothelial dysfunction. They provided evidence that endothelial dysfunction is associated with increases in oxidized and heme-free sGC, both of which cannot be activated by NO. The clinical relevance of their findings was demonstrated in vitro in arterial rings from hypertensive rats, atherosclerotic rabbits, apolipoprotein E−/− mice, and from humans with diabetes, as well as in in vivo models of endothelial dysfunction. Importantly, the novel new NO- and heme-independent sGC activator BAY 58-2667 was more effective and demonstrated increased potency in these pathophysiological and oxidative stress conditions.

BAY 58-2667 (Figure 1) represents a unique drug in that it activates sGC not only independently of NO but also independently of the prosthetic heme group.5 Indeed, BAY 58-2667 preferentially activates the enzyme when the heme iron is oxidized (Fe3+) or the heme moiety is missing, which are 2 conditions that render the enzyme insensitive to both its endogenous ligand NO and exogenous nitrovasodilators.5,6 The concept has been advanced that under physiological conditions there is a pool of oxidized or heme-free sGC other than the reduced and, thus, NO-sensitive sGC, and that this NO-insensitive pool is increased under pathophysiological conditions associated with oxidative stress.5 Because BAY 58-2667 also lacks tolerance development and potentially adverse cGMP-independent actions associated with conventional nitrovasodilators, it appears as a promising novel therapeutic for cardiovascular disease.
Figure 1. Chemical structure of BAY 58-2667.

Congestive heart failure (CHF) represents a state of endotherial dysfunction with excessive systemic and renal vasoconstriction. To date, the cardiorenal actions of direct NO- and heme-independent sGC activation by BAY 58-2667 in CHF remain undefined. We hypothesized that acute activation of the NO-sGC-cGMP pathway by BAY 58-2667 in experimental CHF would result in potent and favorable systemic and renal vasodilation with cardiac unloading while preserving renal function. To test this hypothesis, BAY 58-2667 was acutely infused intravenously in a well established model of severe CHF.

Methods
This study was in accordance with the Animal Welfare Act, and it was approved by the Mayo Clinic Animal Care and Use Committee.

Severe CHF was induced in 7 male mongrel dogs (weight: 20 to 28 kg) by rapid right ventricular pacing at 240 bpm as described previously in detail. On day 11 of pacing, cardiorenal parameters were assessed in an acute study under anesthesia with pentobarbital and fentanyl. The day before the experiment, animals were fed 300 mg of lithium carbonate for the assessment of renal tubular function and were fasted with ad libitum access to water. Animals were intubated and mechanically ventilated with room air and supplemental oxygen (5 L/min). A flow-directed balloon-tipped thermodilution catheter was inserted via the right external jugular vein for hemodynamic measurements, and aortic pressure was assessed via a line inserted via the femoral artery. Cardiac output was assessed by the thermodilution method in triplicate and averaged (Cardiac output model 9510-A computer, American Edwards Laboratories). Via a left lateral flank incision, the left ureter was cannulated for urine collection. An electromagnetic flow probe was placed on the renal artery (Carolina Medical Electronics) to measure renal blood flow. After surgical preparation, inulin (1 mL/min; preceded by a weight-adjusted bolus) and saline (1 mL/min) were continuously administered via lines in the femoral vein. After 60 minutes of equilibration, a 30-minute baseline clearance was done that included urine collection, blood sampling, and hemodynamic measurements. Pressure tracings and renal blood flow were recorded and analyzed digitally (Sonometrics Corporation). After the baseline clearance, the saline infusion was replaced with a first dose of BAY 58-2667 (0.1 µg/kg per minute) and an infusion rate of 1 mL/min. After a lead-in period of 15 minutes, a second 30-minute clearance was done. Subsequently, a second dose of BAY 58-2667 (0.3 µg/kg per minute with an infusion rate of 1 mL/min) was administered, and after 15 minutes of lead-in a third clearance was done. Pacing was suspended for the time of surgical preparation but restarted before the equilibration period and continued throughout the acute protocol. Proximal and distal fractional sodium reabsorption were assessed with the lithium clearance technique. Left ventricular external work in joules per minute was estimated as follows: (mean arterial pressure–pulmonary capillary wedge pressure)×cardiac output×0.13.

Assays
Electrolytes were measured by flame photometry (IL943, Instrumentation Laboratory). Plasma renin activity, aldosterone, and atrial and B-type natriuretic peptide (ANP and BNP, respectively) were measured by radioimmunoassay as described previously. Glomerular filtration rate (GFR) was calculated by inulin clearance. Plasma and urine inulin were measured by the anthrone method.

Statistical Analysis
Values are expressed as mean±SEM. Parameters at baseline and with drug administration were compared with 1-way ANOVA for repeated measures with posthoc Dunn’s test for normally distributed data and Friedman’s test with posthoc Dunn’s test for not normally distributed data. A P<0.05 was considered statistically significant.

Results
Results are presented in the Table and Figure 2.

Cardiovascular Function
Administration of BAY 58-2667 significantly and dose-dependently reduced mean arterial, right atrial, pulmonary artery, and pulmonary capillary wedge pressures (Figure 2). Systemic vascular resistance decreased, whereas cardiac output increased. Left ventricular external work (P=0.42) and pulmonary vascular resistance (P=0.21) remained unchanged. Renal perfusion pressure was significantly decreased.

Renal Function
GFR was maintained (P=0.55). Renal blood flow increased, and renal vascular resistance decreased. Urine flow (P=0.98) and urinary sodium excretion (P=0.22) remained unchanged. Proximal fractional sodium reabsorption decreased significantly, whereas distal fractional sodium reabsorption remained unchanged.

Neurohumoral Function
BAY 58-2667 administration decreased hematocrit significantly. Both ANP and BNP decreased (both P=0.04), consistent with cardiac unloading. There were no significant changes in plasma renin activity (P=0.31) or aldosterone (P=0.19).

Discussion
We report for the first time the cardiorenal actions of a novel NO- and heme-independent sGC activator, BAY 58-2667, in an experimental model of severe CHF. Intravenous administration of BAY 58-2667 resulted in potent systemic and renal vasodilation. This vascular response resulted in cardiac unloading with decreased cardiac filling pressures and an increase in cardiac output, which were paralleled by reductions in circulating ANP and BNP, both markers of myocardial stretch. Despite the reduction in arterial pressure, renal hemodynamic and excretory function was preserved.

In the current study, we used a model of experimental CHF produced by rapid ventricular pacing-induced ventricular dysfunction. This model closely mimics severe overt CHF in humans. Specifically, as in the current study and as reported previously by us and others, this model is characterized by severe systolic dysfunction, ventricular dilation, systemic and renal vasoconstriction, avid sodium and water retention,
neurohumoral activation, oxidative stress, and endothelial dysfunction. In this model of CHF, BAY 58-2667 potently reduced cardiac preload and afterload as indicated by the decreases in right atrial pressure, pulmonary capillary wedge pressure, and mean systemic and pulmonary arterial pressure. Concomitantly, cardiac output was increased. The likely primary mechanism for these effects is vasorelaxation of arteries and veins mediated by sGC-generated cGMP. Because BAY 58-2667 preferentially activates sGC when it is oxidized or heme-free, these findings suggest that a significant pool of these forms is present in experimental HF, which would be consistent with the presence of oxidative stress.

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Although sGC activation with sodium nitroprusside and particulate GC (pGC) activation with ANP have been reported to increase endothelial permeability in some conditions in vitro, there is also evidence to suggest that, for example, the effect of NO depends on the type of stimulation, site of action, and interactions with other cell types. It has also been shown that a cGMP analogue can attenuate the increase in endothelial permeability induced by hydrogen peroxidase. Further studies are needed to characterize the effect of BAY 58-2667 on endothelial permeability in different conditions. The reduction in ANP and BNP can be interpreted as a neurohumoral indication of the beneficial cardiac unloading actions of BAY 58-2667, because cardiac stretch is a major stimulus for the secretion of the 2 cardiac natriuretic peptides. Of note, ANP and BNP via cGMP produced by pGC activation have vasodilating and natriuretic actions and inhibit the secretion of renin and aldosterone. At least theoretically, their reduction could have the opposite effect in this study. Indeed, reciprocal regulation of blood vessels has been reported for sGC and pGC, that is, activation of one system attenuates the response to the other. It remains to be determined whether pGC-induced natriuresis is affected by simultaneous sGC activation, whether oxidized or not. It should also be noted that, whereas both sGC and pGC signal via cGMP, the spatial and temporal distribution of this cGMP signal is important. Indeed, reciprocal regulation of blood vessels has been reported for sGC and pGC, that is, activation of one system attenuates the response to the other. It remains to be determined whether pGC-induced natriuresis is affected by simultaneous sGC activation, whether oxidized or not. It should also be noted that, whereas both sGC and pGC signal via cGMP, the spatial and temporal distribution of this cGMP signal is important. 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with zaprinast increases renin secretion. This effect could be attenuated by inhibition of neuronal NO synthase, thus implicating sGC in this process. Although it remains to be established how BAY 58-2667 affects renin- or aldosterone-secreting cells in heart failure, our study suggests that the net effect of direct and indirect actions is neutral.

Of special importance in CHF is renal function, because impaired GFR has been associated with decreased survival in CHF.23,24 BAY 58-2667 administration significantly reduced renal perfusion pressure secondary to a decrease in arterial pressure. A decrease in renal perfusion pressure would be expected to decrease glomerular hydrostatic pressure and, thus, GFR; however, GFR was maintained with BAY 58-2667. There are at least 2 likely explanations. First, BAY 58-2667 may relax the afferent glomerular arteriole more than the efferent, thus maintaining glomerular hydrostatic pressure. Second, BAY 58-2667 may have increased the coefficient of filtration, which would promote filtration. It should also be noted that proximal tubular reabsorption decreased, although in this avid sodium retaining model overall sodium excretion did not increase. This effect on proximal reabsorption is consistent with the report that sGC is present in human proximal tubular cell and regulates cellular sodium absorption.25,26

An increase in cardiac preload secondary to vasoconstriction and sodium and water retention increases right-sided cardiac pressures contributing to the syndrome of CHF. We reported previously that the NO-independent but heme-dependent sGC stimulator BAY 41-2272 was a potent arterial vasodilator but did not decrease right atrial pressure in the same model of severe CHF.27 In contrast, BAY 58-2667 decreased right atrial pressure, as well as pulmonary capillary wedge pressure. Based on our knowledge of BAY 58-2667 as an NO- and heme-independent sGC activator that stimulates even NO-insensitive oxidized and heme-free sGC, one may conclude that this differential response is suggestive of a higher degree of oxidative stress with a higher prevalence of NO-unresponsive sGC in the venous circulation. Another potential mechanism is that NO activates sGC synergistically with BAY 41-2272 but only additively with BAY 58-2667. If, as has been reported in other studies, endogenous NO production is higher in arteries than in veins, BAY 41-2272 can be expected to be more potent in arteries.28 The differential effect on right atrial pressure provides a rationale for favoring the use of BAY 58-2667 as compared with BAY 41-2272 in CHF. Indeed, clinical studies with BAY 58-2667 in patients with acute decompensated heart failure are currently being conducted.29

We also reported previously the acute cardiovascular effects of intravenous nitroglycerin in experimental CHF, which were almost identical to those seen with BAY 58-2667 in the current study.27 The similar pharmacodynamic profile of BAY 58-2667 and nitroglycerin also suggests that the major beneficial hemodynamic actions of nitrovasodilators are mediated by the sGC–cGMP signaling cascade rather than a cGMP-independent pathway, because BAY 58-2667, through its novel mechanism, lacks non-cGMP actions to our knowledge.

Despite the similarities after acute administration of BAY 58-2667 and nitroglycerin, there are important differences between the 2 compounds, which should continue to be defined in future experimental and human studies. First, nitroglycerin, like other organic nitrates, is associated with the development of tolerance with chronic administration. In contrast, because of its unique mechanism of action, chronic administration of BAY 58-2667 has been reported in experimental hypertension not to result in tolerance. Indeed, it even relaxes isolated artery rings from normal and nitrate-tolerant rabbits with similar potency.5 However, the long-term efficacy of chronic BAY 58-2667 in heart failure still needs to be defined, because it may be reduced by potential increases in cGMP-degrading phosphodiesterase activity, downregulation or desensitization of sGC and signaling molecules further downstream, or other counteracting mechanisms, such as fluid retention. Second, nitroglycerin needs to be bioactivated to stimulate sGC. This process can increase oxidative stress.
nitrovasodilators, sGC stimulators (eg, BAY 41-2272), and sGC activators (eg, BAY 58-2667). Nitrovasodilators only stimulate NO-sensitive sGC, which contains the heme moiety with a ferrous ion (Fe$^{2+}$); furthermore, nitrovasodilators have cGMP-independent actions. In contrast, BAY 41-2272 solely activates the NO-sensitive sGC without the cGMP-independent actions of nitrovasodilators. BAY 58-2667 activates preferentially oxidized (Fe$^{3+}$) or heme-free sGC, which are both insensitive to NO. The question marks indicate that little is known about the transition between the different forms, their prevalence in health and disease, and their potential restoration to the NO-sensitive form.

Figure 3. Schematic illustrating the differences among nitrovasodilators, sGC stimulators (eg, BAY 41-2272), and sGC activators (eg, BAY 58-2667). Nitrovasodilators only stimulate NO-sensitive sGC, which contains the heme moiety with a ferrous ion (Fe$^{2+}$); furthermore, nitrovasodilators have cGMP-independent actions. In contrast, BAY 41-2272 solely activates the NO-sensitive sGC without the cGMP-independent actions of nitrovasodilators. BAY 58-2667 activates preferentially oxidized (Fe$^{3+}$) or heme-free sGC, which are both insensitive to NO. The question marks indicate that little is known about the transition between the different forms, their prevalence in health and disease, and their potential restoration to the NO-sensitive form.

by decreasing antioxidants, such as thiols. Nitroglycerin has been reported to inhibit mitochondrial aldehyde dehydrogenase, increase mitochondrial production of reactive oxygen species, and impair relaxation to acetylcholine. Moreover, nitroglycerin can lead to the generation of peroxynitrite, thus promoting oxidative stress with oxidation of sGC and further aggravation of endothelial dysfunction.3,32 Furthermore, it appears that oxidation of sGC and removal of the heme moiety promotes degradation of sGC, whereas binding of BAY 58-2667 inhibits degradation of sGC.4 Lastly, the biological actions of endogenous NO and nitrovasodilators are mediated not only by cGMP, but also by cGMP-independent mechanisms, such as modification of proteins by S-nitrosothiol bond formation, which may be beneficial or detrimental.33 In contrast, cGMP via its direct actions on vascular function and via modulation of the expression of various genes has been implicated in opposing the pathophysiology of hypertension, cardiac hypertrophy, and atherosclerosis.34 Figure 3 contrasts the mechanisms of nitrovasodilators, BAY 41-2272, and BAY 58-2667, suggesting that a more selective activation of cGMP may be possible with the latter 2 drugs.

Whereas in this study we provide a comprehensive investigation of the cardiorenal actions of acute administration of BAY 58-2667 in CHF, others have investigated the effects of chronic BAY 58-2667 in other disease models. Dumitrascu et al35 reported that BAY 58-2667 reversed hemodynamic and structural changes in mice and rats with experimental pulmonary arterial hypertension. Kalk et al36 reported that BAY 58-2667 in a 5/6 nephrectomy model in rats reduced blood pressure and left ventricular hypertrophy and attenuated renal disease progression. In both studies, BAY 58-2667 showed hemodynamic and antiremodeling actions without an indication for tolerance development. These findings underscore the potential of BAY 58-2667 as a therapeutic in cardiorenal disease states.

**Perspectives**

We report for the first time that the novel NO- and heme-independent sGC activator BAY 58-2667 has potent systemic and renal vasodilating actions, unloads the heart, increases cardiac output, and preserves GFR and sodium and water excretion in experimental CHF. Unlike conventional nitrovasodilators, BAY 58-2667 is a selective sGC activator without cGMP-independent actions and is not associated with the development of tolerance, providing a novel pharmacological opportunity to treat endothelial dysfunction. Further studies are clearly warranted to explore the potential of this new therapeutic approach for the acute and chronic treatment of heart failure.

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**Disclosures**

J.-P.S. is employed by Bayer HealthCare AG, Germany. He has filed several patents concerning sGC activators. H.L. is the principal investigator of a clinical study using BAY 58-2667 in patients with acute decompensated heart failure. He does not receive any remuneration for this role. The remaining authors report no conflicts.

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